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The purpose of this proposal is to evaluate the role of IQGAP1 in neoplastic transformation and metastasis of breast epithelial cells. The main emphasis is on whether IQGAP is involved in invasion and metastasis of transformed breast epithelial cells, as well as the possible involvement of IQGAP1 in regulating b-catenin function. Major findings to date are: (i) there is a high level of expression of IQGAP1 in breast epithelium; and (ii) overexpression of IQGAP1 in mammalian cells enhances cell migration and proliferation. These data reveal that IQGAP1 has a fundamental role in cell motility and invasion in breast epithelium. This information could have potential therapeutic implications in patients with breast cancer.

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INTRODUCTION

Modulation of cell adhesion and motility are critical factors affecting malignancy. A role for IQGAP1 in neoplasia is suggested by evidence that implicates IQGAP1 in cell-cell adhesion and organization of the actin cytoskeleton. IQGAP1 appears to act as a focal point of cross-talk where diverse regulatory and structural proteins converge, suggesting that IQGAP1 is a critical link in the signal transduction pathways leading to enhancement of cell motility and altered cell-cell adhesion. Importantly, IQGAP1 is significantly increased in highly metastatic melanoma and gastric cells. The hypothesis to be evaluated in this proposal is that elevated levels of IQGAP1 promote neoplastic transformation and metastasis. The role of IQGAP1 in cell motility and proliferation will be examined. In addition, IQGAP1 also interacts with β-catenin, a proto-oncogene that participates in cell-cell adhesion and transcriptional co-activation. The effects of IQGAP1 on the stability, subcellular localization and transcriptional co-activation of β-catenin will be assessed. Elucidation of the role played by IQGAP1 in promoting neoplasia and metastasis will enhance our comprehension of the molecular mechanisms responsible for breast cancer.

BODY

Research accomplishments are described according to the Tasks listed in the approved Statement of Work. These results encompass the period 8th January 2003 to 30th November 2003.

Task 1. Ascertain whether IQGAP1 is involved in invasion and metastasis of transformed cells

i. Examine IQGAP1 protein levels in cell types of varying malignancy

Our studies involving analysis of selected cell lines revealed that there is a very high level of expression of IQGAP1 in breast epithelium (Fig. 1). Due to the very exciting and potentially significant observations on the role of IQGAP1 in tumour cell migration (see Task 1, section ii, below), the planned studies to examine the relative levels of expression of IQGAP1 protein in multiple human breast carcinoma cell lines – MCF-7, ZR-75-1, T-47D, MDA-MB-231, 21T and Hs578T – and non-tumorigenic MCF-10 and MTSV-1 has not yet been completed. These experiments are ongoing. In addition, our collaborator, Richard Hynes at the Center for Cancer Research at MIT observed **that** IQGAP1 concentrations in metastatic melanoma cells are higher than those in non metastatic cells (data not shown). These findings support the hypothesis that highly metastatic cells express increased IQGAP1 protein.

ii. Determine if IQGAP1 overexpression promotes tumour cell migration

Migration of MCF-7 cells stably transfected with pcDNA3 vector (termed MCF/V cells) and MCF-7 cells stably transfected with pcDNA3-IQGAP1 (termed MCF/I cells) expressing IQGAP1 at three times the levels expressed in MCF/V cells was

evaluated. Motility of MCF/V and MCF/I cells through Transwell pores coated with human collagen I was quantified by counting fields of migratory cells under a light microscope. MCF/I cells exhibited a 2.82 ± 0.17-fold (mean ± S.E., n=16, p<0.005) greater motility than MCF/V cells (Fig. 2). Similarly, transient overexpression of IQGAP1 accelerated motility by 1.60 ± 0.07-fold (n=4, p<0.005) and 1.67 ± 0.09-fold (n=4, p<0.005) in HEK-293H cells and highly motile MDA-MB-231 cells, respectively (Fig. 3). Because of this very exciting discovery, considerable effort was directed toward this task. The role of IQGAP1 in increased cell motility implied that IQGAP1 could also contribute to cell invasion. For invasion assays, motility of cells through Matrigel-coated Transwells was monitored. Our results reveal that MCF/I cells were 2.5-fold more invasive than MCF/V cells (Fig. 4).

iii. Determine if IQGAP1 overexpression promotes tumour cell proliferation

Studies have been initiated to address this task. Equal numbers of MCF/V and MCF/I cells were examined for their [^{3}H]thymidine uptake to measure DNA synthesis and therefore, cell proliferation. Initial analysis reveals that, compared to MCF/V cells, MCF/I cells increased [^{3}H]thymidine uptake (data not shown). Further analysis is scheduled to commence in the second year of funding as originally proposed.

iv. Develop antisense oligonucleotides to inhibit IQGAP1 expression and analyze the effect of decreased IQGAP1 protein levels on cell migration and proliferation

These studies are scheduled to commence in the second year of funding as originally proposed.

Task 2. Test the hypothesis that IQGAP1 regulates β-catenin function in breast cancer cell lines of varying malignancy

This task is scheduled to commence in the second year of funding as originally proposed.

KEY RESEARCH ACCOMPLISHMENTS

- there is a high level of expression of IQGAP1 in breast epithelium
- overexpression of IQGAP1 in mammalian cells enhances cell migration
- IQGAP1 overexpressing MCF-7 cells are more invasive than vector-transfected MCF-7 cells
- overexpression of IQGAP1 enhances cellular proliferation

REPORTABLE OUTCOMES

None

CONCLUSIONS

The work performed to date has yielded some insights into the role of IQGAP1 in cell motility. We observed that there is a high level of IQGAP1 protein expressed in breast epithelium. In addition, overexpression of IQGAP1 in transformed breast cells enhanced cell migration and cell proliferation. Initial findings support the hypothesis that IQGAP1 plays a significant role in the metastasis of breast epithelial cells. This information could help determine whether IQGAP1 is a potential therapeutic target for breast cancer.

REFERENCES

None

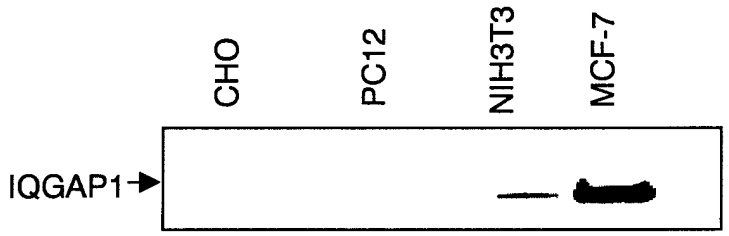

Fig. 1. Comparison of the amount of IQGAP1 among cells. Equal amounts of protein lysate from the indicated cell lines were analyzed for IQGAP1 by Western blotting.

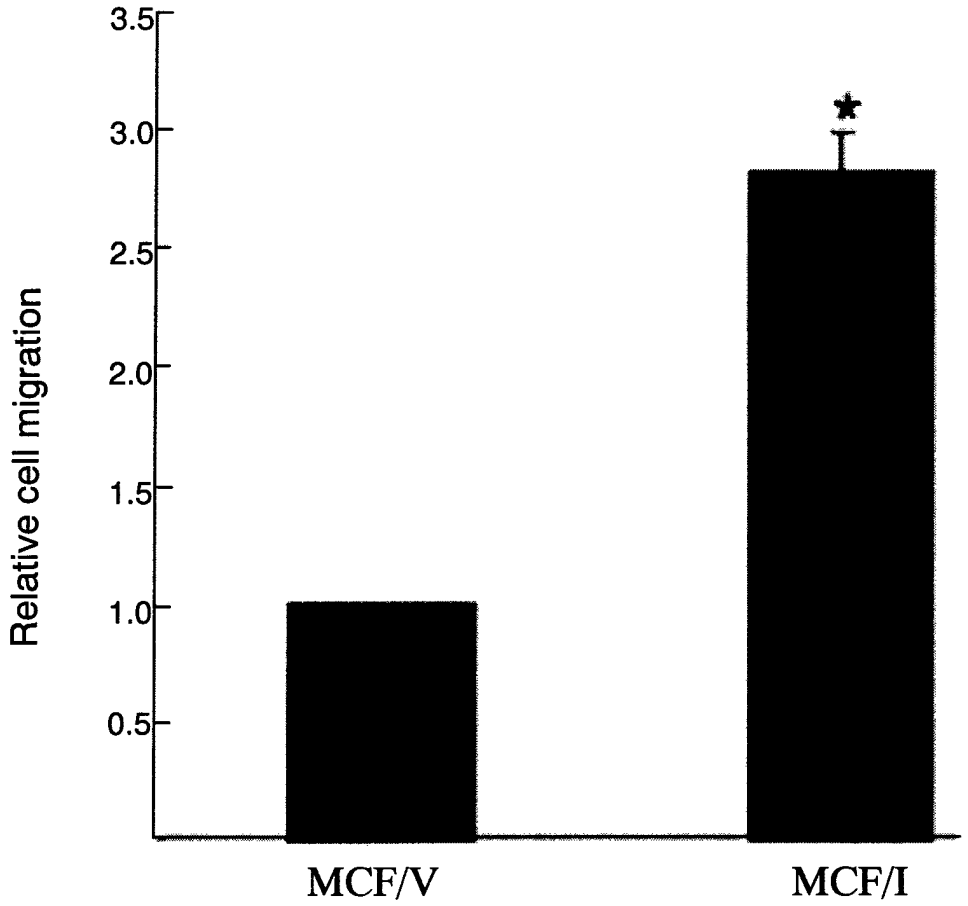

Fig. 2. Migration of MCF/V and MCF/I cells through Transwell pores was quantified by counting fields of migratory cells under a light microscope. Data, expressed relative to migration of MCF/V cells, represent the means ± S.E. (n = 16). *, significantly different from MCF/V ($p < 0.005$).

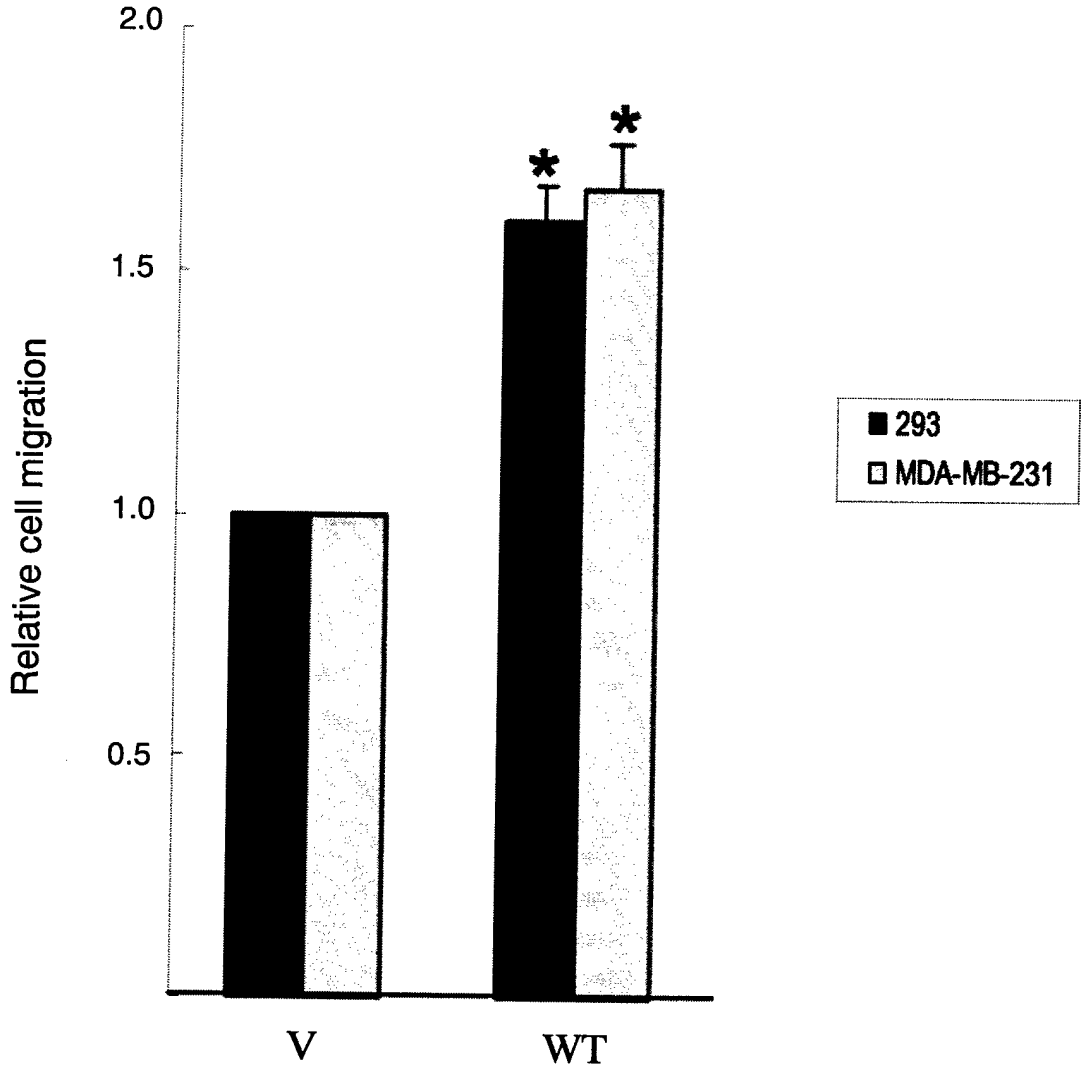

Fig.3. Migration through Transwells of HEK-293H and MDA-MB-231 cells transiently transfected with vector (*V*) or IQGAP1 (*WT*) was examined. Data are expressed relative to the migration of vector-transfected cells and represent the means ± S.E. ($n = 4$). *, significantly different from vector-transfected cells ($p < 0.005$).

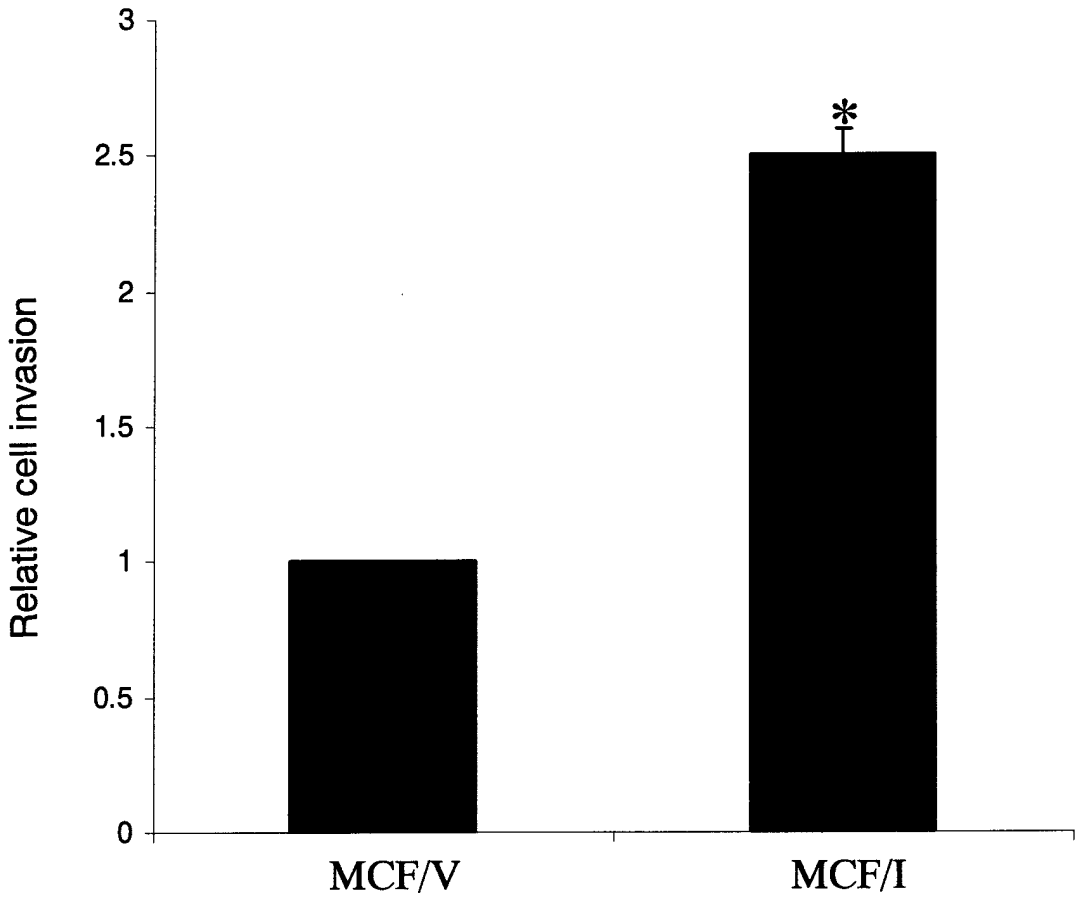

Fig. 4. Invasion of MCF/V and MCF/I cells was quantified by counting fields of migratory cells under a light microscope. Data, expressed relative to migration of MCF/V cells, represent the means ± S.E. (n = 4). *, significantly different from MCF/V ($p < 0.005$).